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# Evaluation of EMIT Adapted to the ⟨Cobas⟩ Bio Centrifugal Analyzer

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**Summary:** EMIT assays for the determination of phenytoin, methotrexate, disopyramide, digoxin and thyroxine were adapted to the Cobas Bio centrifugal analyzer and compared with the corresponding laboratory routine procedures. Evaluation of the data from the Cobas Bio by 5 different mathematical models showed that the four-parameter logit model correlated best with the comparison procedures and the originally recommended calculation model for the reagent lots used in our study. The precision of the EMIT phenytoin, methotrexate and disopyramide assays was in most cases very good (between-days coefficients of variation 1.6–7.5%). A lower precision was observed with the EMIT digoxin assay (between-days coefficient of variation 9.2–16.8%) and at low concentrations also with the EMIT thyroxine assay (3.1–21.4%). Calibration curves of the EMIT phenytoin and methotrexate assays were stable for at least one hour. The results from the determination of phenytoin, methotrexate and disopyramide in patient samples by use of the Cobas Bio were in good agreement with those values obtained with the EMIT/LAB. The data determined with the EMIT digoxin assay adapted to the Cobas Bio correlated better with those of a radioimmunoassay than the values measured with the EMIT/LAB system. The results of thyroxine determinations with EMIT by use of the Cobas Bio and the original procedure with an ABA-100 were in good agreement and on average about 12% lower than those measured by radioimmunoassay. The Cobas Bio allows rapid determinations with EMIT and a reduction in direct costs of up to 85%.

*Erprobung von EMIT nach Adaptation an den Cobas Bio Zentrifugalanalysator*

**Zusammenfassung:** EMIT Tests zur Bestimmung von Phenytoin, Methotrexat, Disopyramid, Digoxin und Thyroxin wurden an den Cobas Bio Zentrifugalanalysator adaptiert und mit entsprechenden Methoden verglichen, welche gegenwärtig als Routineverfahren eingesetzt werden. Die Auswertung der Daten vom Cobas Bio mit 5 verschiedenen mathematischen Modellen zeigte, daß das „four-parameter logit" Modell für die in unserer Studie verwendeten Reagentienchargen am besten mit den Vergleichsverfahren und dem ursprünglich empfohlenen Rechenmodell korrelierte. Die Präzision der EMIT Phenytoin, Methotrexat und Disopyramid Tests war in den meisten Fällen sehr gut (Variationskoeffizienten von Tag zu Tag 1.6–7.5%). Eine niedrigere Präzision wurde mit dem EMIT Digoxin Test (Variationskoeffizienten von Tag zu Tag 9.2–16.8%) und bei geringen Konzentrationen auch mit dem EMIT Thyroxin Test (3.1–21.4%) beobachtet. Die Kalibrierkurven des EMIT Phenytoin und Methotrexat Tests waren über mindestens eine Stunde stabil. Die Ergebnisse der Bestimmung von Phenytoin, Methotrexat und Disopyramid in Patientenproben mit Hilfe des Cobas Bio stimmten gut mit den Werten überein, welche mit dem EMIT/LAB erhalten wurden. Die Daten, welche mit dem an den Cobas Bio adaptierten EMIT Digoxin Test bestimmt wurden, korrelierten besser mit denen eines Radioimmunotests als mit den am EMIT/LAB System gemessenen Werten. Die Ergebnisse von Thyroxinbestimmungen mit EMIT am Cobas Bio stimmten gut mit denen des Originalverfahrens am ABA-100 überein und waren durchschnittlich um etwa 12% niedriger als die mit dem Radioimmunotest gemessenen Werte. Der Cobas Bio gestattet rasche Bestimmungen mit EMIT und eine Reduktion der direkten Kosten um bis zu 85%.

## Introduction

In recent years the Enzyme Multiplied Immunoassay Technique (EMIT®) has been adapted to a great number of partially and fully mechanized analytical systems (1). A far-reaching mechanization of the EMIT appears to be necessary in order to achieve high reliability and good practicability with these assays. Furthermore the costs for reagents and technician time can be con-

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siderably reduced if suitable mechanization is chosen. In the present study we report on an evaluation of various EMIT assays adapted to the Cobas Bio centrifugal analyzer.

## Materials and Methods

### Enzyme immunoassay (EMIT)

The reagents for the enzyme immunoassay (EMIT) were obtained from E. Merck (D-6100 Darmstadt):

| Merckotest®-Emit® Reagents | Order No. | Lot No. |
|---|---|---|
| 1. aed Phenytoin | 448307 | KO 2 |
| 2. and Methotrexate | 448355 | KO 4 |
| 3. cad Disopyramide | 448350 | KO 1 |
| 4. cad Digoxin manual | 448314 | KO 1 |
| 5. tfg R-Thyroxine test | 448330 | KO 3 |
| 6. tfg ABA-Thyroxine test | 448328 | KO 5 |

The EMIT was mechanized by use of a ‹Cobas› Bio centrifugal analyzer according to the instructions of the manufacturer of this analytical system (F. Hoffman-La Roche AG, Diagnostica, CH-4002 Basel).

In addition, the EMIT phenytoin, methotrexate, disopyramide, and digoxin assays were performed by the original procedures using an EMIT/LAB system, and thyroxine was determined with EMIT by use of an ABA-100. Furthermore phenytoin and methotrexate determinations were performed by EMIT with an Eppendorf analyzer 5010 (2, 3).

### Radioimmunoassay

Digoxin determinations were carried out by radioimmunoassay using coated tubes (Becton-Dickinson, D-6900 Heidelberg), according to the instructions of the manufacturer. Thyroxine was measured by radioimmunoassay as previously described (4).

### Evaluation of the results by mathematical models

For the evaluation of the results obtained by EMIT with the ‹Cobas› Bio five mathematical models (5, 6) were used:

Model 1: four-parameter logit (the log-logit model)

$$R = Ro + K_c \frac{1}{1+\exp[-(a+b\ln C)]}$$

Model 2: five-parameter logit

$$R = R_o + K_c \frac{1}{1+\exp[-(a+b\ln C+cC)]}$$

Model 3: five-parameter exponential

$$R = R_o + K \exp[a\ln C + b(\ln C)^2 + c(\ln C)^3]$$

Model 4: five-parameter polynominal

$$\ln C = a + b\frac{(R-R_o)}{100} + c\frac{(R-R_o)^2}{100} + d\frac{(R-R_o)^3}{100}$$

Model 5: spline approximation

$$c = a_i + b_i(R-R_i) + c_i(R-R_i)^2 + di(R-R_i)^3$$

with a set of parameters $a_i$, $b_i$, $c_i$, and $d_i$ for each interval between the rates for two successive standards $R_i$ and $R_{i+1}$,

Where:

| | |
|---|---|
| R | = rate of change of absorbance |
| C | = concentration of the standards |
| $R_o$ | = the predicted rate for a standard with zero concentration |
| $K_c$ | = the predicted difference between $R_m$, the rate of absorbance change for a standard with infinite concentration, and $R_o$ |
| K | = a scale parameter for model 3 |
| a, b, c, d | = various parameters which define the non-linear elements of each model |

## Results

### Precision

The EMIT phenytoin assay yielded between-days coefficients of variation of 2.2–5.3%, if the actual calibration curve of each run was used and of 3.9–7.0% with a fixed calibration curve (fig. 1). The deviation of means from target values of the corresponding control

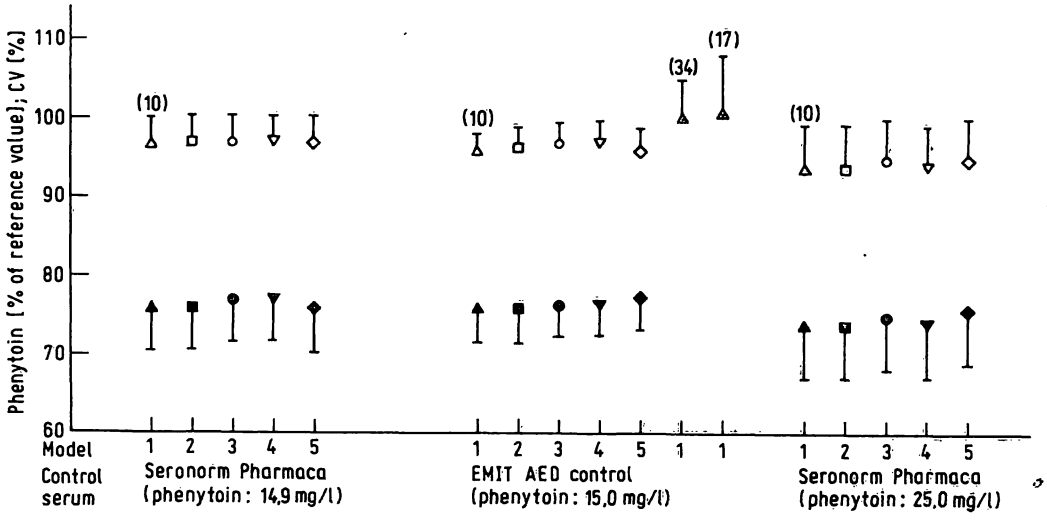


Fig. 1. Between-days precision of the EMIT phenytoin assay adapted to the ‹Cobas› Bio centrifugal analyzer. The mean values in % of the phenytoin reference values in various control sera and the coefficients of variation (vertical bars) with the number of contributing results in parenthesis are given. Results were evaluated by various mathematical methods using the actual calibration curve of each run (model: 1△, 2□, 3○, 4▽, 5◇), a single fixed calibration curve of the very first run (filled symbols) and the calibration curve of the first run from each day at a time interval to the actual run of up to 60 minutes (▲).

sera was only 3–6%, if the actual calibration curve of each run was used, but 23–26% with a fixed calibration curve. No distinct difference in precision was observed with the various mathematical models used for evaluation of the results.

When phenytoin determinations were routinely performed on the ⟨Cobas⟩ Bio over a period of 34 days no deviation of the mean value from the target value of the control serum was observed and the coefficient of variation was 5.0%. Evaluation of the results obtained in a second daily run (at a time interval of up to 60 minutes) by use of the first calibration curve of each day yielded a deviation of the mean value from the target value of less than 1% and a coefficient of variation of 7.5% (fig. 1).

Within series the coefficient of variation was 1.7% (mean value: 13.6 mg/l, n = 20), if a sample volume of 4 µl was used. The coefficient of variation was 1.6% and 3.0% at a sample size of 3 µl and 5 µl respectively.

With the EMIT methotrexate assay between-days coefficients of variation were (fig. 2) 3.4–5.5% (calibration curve for each run) and 4.0–6.6% (fixed calibration curve). The deviations of means from the target values were less than 10% with accompanying calibration curves and up to 17% with a fixed calibration curve. Routine determinations of methotrexate over a period of 32 days yielded no relevant deviation of the mean value from the target value and a coefficient of variation of 3.9% in the medium measurement range.

The use of the first calibration curve of each day instead of an actual calibration curve yielded similar results with a slightly higher coefficient of variation of 6.9%. Again no distinct differences in precision were observed with the mathematical models tested.

With the EMIT disopyramide assay between-days coefficients of variation ranged from 1.6–3.8% (accompanying calibration curves) and from 6.3–8.1% (fixed calibration curves). The means showed only minor deviations from the target values (up to 6%) with accompanying calibration curves, and strong deviations (up to 24%) with fixed calibration curves (fig. 3).

Between-days coefficients of variation ranged with the EMIT Digoxin manual assay from 9.2–16.8% and with the EMIT® Thyroxine test from 3.1–21.4% (figs. 4 and 5).

In all of these assays the precision was almost equal whichever mathematical model was used for the evaluation of the results. From our experience it seems inadvisable to use a fixed calibration curve over a period of several days. Perhaps better calibration curves for this purpose can be obtained if the data of several calibration curves of the same lot are averaged. Within the same working day the stability of the calibration curves of the EMIT phenytoin and methotrexate assays was sufficient for at least one hour.

## Method comparison

Several patients' sera were analyzed with the ⟨Cobas⟩ Bio and the EMIT/LAB or other systems available in the laboratory. The data from the ⟨Cobas⟩ Bio were evaluated by 5 different mathematical models to find out which one correlated best with the comparison pro-

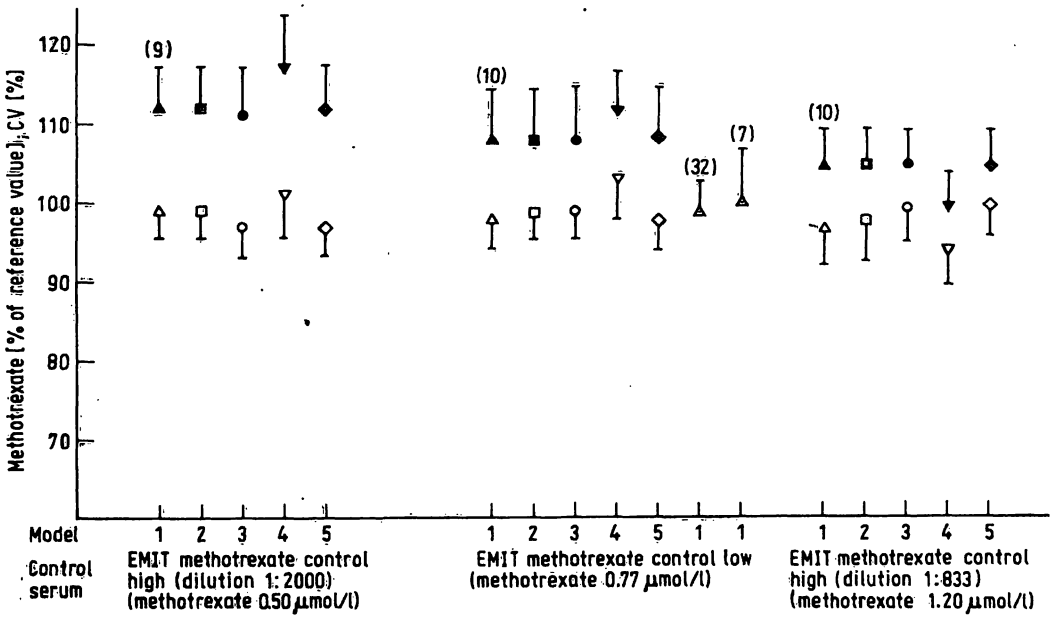


Fig. 2. Between-days precision of the EMIT methotrexate assay adapted to the ⟨Cobas⟩ Bio centrifugal analyzer. For further explanation see legend of figure 1.

cedures and the originally recommended calculation model. Evaluation was based on the significance of the bias ($\bar{x} - \bar{y}$, paired t-test), the standard error of the $y_i$-values and of the residuals $s_{y.x}$ which is a measure of the distribution of the y-component about the standardized principal component.

*Phenytoin*

The data from the determination of the phenytoin concentration are summarized in table 1. In the first 5 lines the ⟨Cobas⟩ Bio data are compared with those of the EMIT/LAB system. The mean values between both

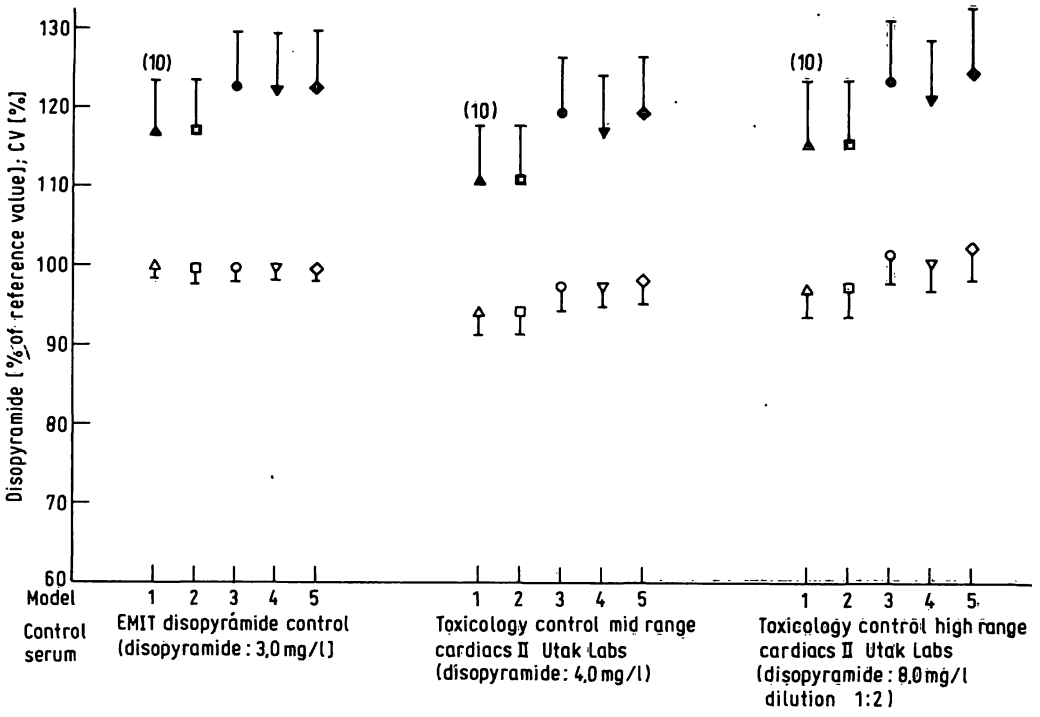


Fig. 3. Between-days precision of the EMIT disopyramide assay adapted to the ⟨Cobas⟩ Bio centrifugal analyzer. For further explanation see legend of figure 1.

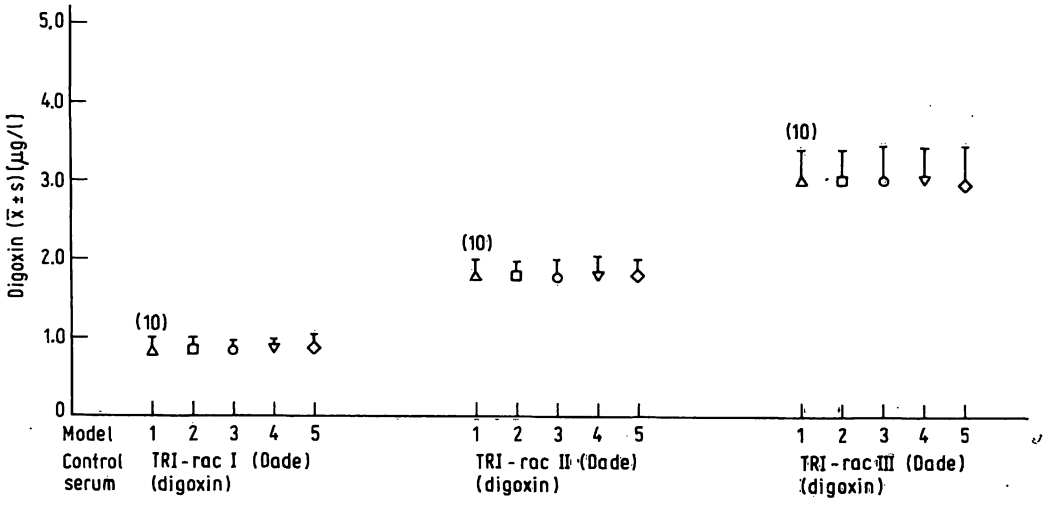


Fig. 4. Between-days precision of the EMIT digoxin assay adapted to the ⟨Cobas⟩ Bio centrifugal analyzer. The mean values and standard deviations (vertical bars) are given (n = 10). Results were evaluated by various mathematical methods using the actual calibration curve of each run (model: 1△, 2□, 3○, 4▽, 5◇). With the control sera used no reference values were available for EMIT.

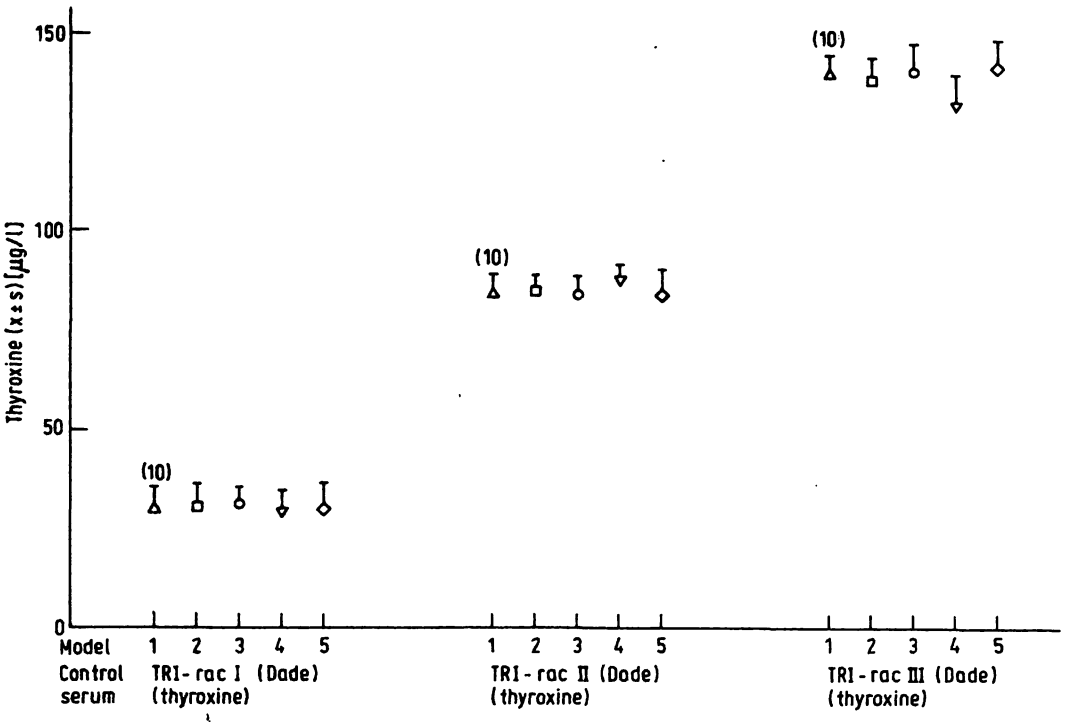


Fig. 5. Between-days precision of the EMIT thyroxine assay adapted to the ⟨Cobas⟩ Bio centrifugal analyzer. For further explanation see legend of figure 4.

Tab. 1. Comparison of the results obtained by the EMIT phenytoin assay mechanized with a ⟨Cobas⟩ Bio centrifugal analyzer, an EMIT/LAB system, and an Eppendorf analyzer 5010 in samples from patients. Various mathematical models were used for evaluation of the results obtained by the ⟨Cobas⟩ Bio.

| Methods (y vs x) | n[2] | Standardized principal component Slope | Intercept | $s_{y.x}$[3] | $\bar{y}$ (s)[4] | $\bar{x}$ (s) | t[5] | Correlation coefficient |
|---|---|---|---|---|---|---|---|---|
| ⟨Cobas⟩ Bio (1)[1]) vs EMIT/LAB | 50 | 0.95 | 0.38 | 0.60 | 9.65 (5.37) | 9.76 (5.66) | 0.89 | 0.987 |
| ⟨Cobas⟩ Bio (2) vs EMIT/LAB | 50 | 0.95 | 0.37 | 0.60 | 9.64 (5.37) | 9.76 (5.66) | 0.93 | 0.987 |
| ⟨Cobas⟩ Bio (3) vs EMIT/LAB | 50 | 0.96 | 0.26 | 0.59 | 9.66 (5.45) | 9.76 (5.66) | 0.84 | 0.988 |
| ⟨Cobas⟩ Bio (4) vs EMIT/LAB | 50 | 0.96 | 0.29 | 0.59 | 9.67 (5.44) | 9.76 (5.66) | 0.71 | 0.988 |
| ⟨Cobas⟩ Bio (5) vs EMIT/LAB | 50 | 0.96 | 0.27 | 0.60 | 9.68 (5.45) | 9.76 (5.66) | 0.68 | 0.987 |
| ⟨Cobas⟩ Bio (1) vs Eppendorf 5010 | 50 | 1.03 | − 0.27 | 0.56 | 9.65 (5.37) | 9.65 (5.23) | 0.05 | 0.989 |
| ⟨Cobas⟩ Bio (2) vs Eppendorf 5010 | 50 | 1.03 | − 0.28 | 0.56 | 9.64 (5.37) | 9.65 (5.23) | 0.10 | 0.989 |
| ⟨Cobas⟩ Bio (3) vs Eppendorf 5010 | 50 | 1.04 | − 0.40 | 0.58 | 9.66 (5.45) | 9.65 (5.23) | 0.05 | 0.988 |
| ⟨Cobas⟩ Bio (4) vs Eppendorf 5010 | 50 | 1.04 | − 0.37 | 0.58 | 9.67 (5.44) | 9.65 (5.23) | − 0.19 | 0.989 |
| ⟨Cobas⟩ Bio (5) vs Eppendorf 5010 | 50 | 1.04 | − 0.38 | 0.59 | 9.68 (5.45) | 9.65 (5.23) | − 0.20 | 0.988 |

[1]) mathematical model used in parenthesis [2]) number of contributing values [3]) standard error of the residuals
[4]) mean value with standard deviation in parenthesis [5]) t-value (paired t-test)

procedures did not differ significantly. The standard deviation $s_y$ was slightly lower with model 1 and 2. This constellation was observed in the last 5 lines comparing the ⟨Cobas⟩ Bio data with those obtained with the Eppendorf analyzer 5010. Under these conditions $s_{y.x}$ was also slightly lower than with the other models. In conclusion, for the determination of the phenytoin concentration all models provided data in very good agreement.

*Methotrexate*

In comparison with the EMIT/LAB system the mean values from the ⟨Cobas⟩ Bio for the determination of the methotrexate concentration did not significantly differ. The mean values with the Eppendorf analyzer 5010 are significantly about 10% higher than those from the ⟨Cobas⟩ Bio and EMIT/LAB (tab. 2). Again the $s_y$ and $s_{y.x}$ values were lower for the evaluation

Tab. 2. Comparison of the results obtained by the EMIT methotrexate assay mechanized with a ⟨Cobas⟩ Bio centrifugal analyzer, an EMIT/LAB system, and an Eppendorf analyzer 5010 in samples from patients. Various mathematical models were used for evaluation of the results obtained by the ⟨Cobas⟩ Bio.

| Methods (y vs x) | n[2] | Standardized principal component Slope | Intercept | $s_{y.x}$[3] | $\bar{y}$ (s)[4] | $\bar{x}$ (s) | t[5] | Correlation coefficient |
|---|---|---|---|---|---|---|---|---|
| ⟨Cobas⟩ Bio (1)[1] vs EMIT/LAB | 50 | 0.98 | 0.01 | 0.43 | 0.54 (0.32) | 0.54 (0.32) | 0.11 | 0.980 |
| ⟨Cobas⟩ Bio (2) vs EMIT/LAB | 50 | 0.99 | 0.01 | 0.43 | 0.54 (0.32) | 0.54 (0.32) | 0.26 | 0.980 |
| ⟨Cobas⟩ Bio (3) vs EMIT/LAB | 50 | 1.02 | −0.01 | 0.46 | 0.54 (0.33) | 0.54 (0.32) | 0.06 | 0.979 |
| ⟨Cobas⟩ Bio (4) vs EMIT/LAB | 50 | 1.01 | −0.01 | 0.48 | 0.54 (0.33) | 0.54 (0.32) | 0.20 | 0.977 |
| ⟨Cobas⟩ Bio (5) vs EMIT/LAB | 50 | 1.02 | −0.01 | 0.48 | 0.54 (0.33) | 0.54 (0.32) | 0.35 | 0.978 |
| ⟨Cobas⟩ Bio (1) vs Eppendorf 5010 | 50 | 1.00 | −0.06 | 0.36 | 0.54 (0.32) | 0.60 (0.32) | 7.43* | 0.986 |
| ⟨Cobas⟩ Bio (2) vs Eppendorf 5010 | 50 | 1.00 | −0.06 | 0.37 | 0.54 (0.32) | 0.60 (0.32) | 7.24* | 0.986 |
| ⟨Cobas⟩ Bio (3) vs Eppendorf 5010 | 50 | 1.03 | −0.08 | 0.41 | 0.54 (0.33) | 0.60 (0.32) | 6.80* | 0.984 |
| ⟨Cobas⟩ Bio (4) vs Eppendorf 5010 | 50 | 1.03 | −0.08 | 0.40 | 0.54 (0.33) | 0.60 (0.32) | 7.34* | 0.984 |
| ⟨Cobas⟩ Bio (5) vs Eppendorf 5010 | 50 | 1.04 | −0.08 | 0.42 | 0.54 (0.33) | 0.60 (0.32) | 6.32* | 0.983 |

[1]) mathematical model used in parenthesis [2]) number of contributing values [3]) standard error of the residuals
[4]) mean value with standard deviation in parenthesis [5]) t-value (paired t-test)
* significance of the bias $\bar{x} - \bar{y}$ ($p < 0.05$)

method 1 and 2, with the lowest value for method 1. The slope of the principal component was also closer to 1.00 with method 1 and 2. The most unfavorable results were obtained with method 5. In conclusion method 1 (and perhaps 2) appeared to be best suited for the determination of methotrexate concentrations with the ⟨Cobas⟩ Bio.

*Disopyramide*

In comparison with the mean values from the EMIT/LAB the data obtained with the ⟨Cobas⟩ Bio are about 3% lower (tab. 3) for the determination of the disopyramide concentration. This difference is statistically significant with evaluation method 1, 2 and 3 (paired t-test). Here again, the $s_y$ and $s_{y.x}$ values are lowest for method 1 and 2, and highest for method 5.

*Digoxin*

In table 4 the data obtained with ⟨Cobas⟩ Bio are compared with a radioimmunological technique and the EMIT/LAB system. The mean values from the ⟨Cobas⟩ Bio lie in between those measured with both other procedures. These differences are statistically significant (paired t-test).

In this example $s_y$ was lowest with the evaluation method 4, however, from the $s_{y.x}$ values no distinct preference can be derived. The mean value was highest with method 1 which, therefore, may agree best with the EMIT/LAB system.

*Thyroxine*

In the first 5 lines of table 5 the data from the ⟨Cobas⟩ Bio are compared with those obtained with an ABA-100. The mean values between both procedures did not differ significantly (paired t-test). The standard deviation $s_y$ and $s_{y.x}$ are lower for evaluation method 4 and again highest for method 5. The slope b was close to 1.00 only with method 4. Therefore with method 4 the best correlation with the ABA-100 procedure is observed. Next to method 4, method 1 gave the most favorable data. In comparison with a radioimmunoassay the results are approximately 12% lower with the ⟨Cobas⟩ Bio and the ABA-100.

Tab. 3. Comparison of the results obtained by the EMIT disopyramide assay mechanized with a ⟨Cobas⟩ Bio centrifugal analyzer and an EMIT/LAB system in samples from patients. Various mathematical models were used for evaluation of the results obtained by the ⟨Cobas⟩ Bio.

| Methods (y vs x) | n[2] | Standardized principal component Slope | Intercept | $s_{y.x}$[3] | $\bar{y}$ (s)[4] | $\bar{x}$ (s) | t[5] | Correlation coefficient |
|---|---|---|---|---|---|---|---|---|
| ⟨Cobas⟩ Bio (1)[1] vs EMIT/LAB | 21 | 0.93 | 0.08 | 0.08 | 2.42 (0.84) | 2.51 (0.90) | 1.89* | 0.974 |
| ⟨Cobas⟩ Bio (2) vs EMIT/LAB | 21 | 0.93 | 0.08 | 0.08 | 2.42 (0.84) | 2.51 (0.90) | 1.89* | 0.974 |
| ⟨Cobas⟩ Bio (3) vs EMIT/LAB | 21 | 1.02 | −0.16 | 0.12 | 2.41 (0.92) | 2.51 (0.90) | 1.94* | 0.970 |
| ⟨Cobas⟩ Bio (4) vs EMIT/LAB | 21 | 0.98 | −0.02 | 0.09 | 2.44 (0.88) | 2.51 (0.90) | 1.56 | 0.973 |
| ⟨Cobas⟩ Bio (5) vs EMIT/LAB | 21 | 1.07 | −0.24 | 0.14 | 2.43 (0.96) | 2.51 (0.90) | 1.45 | 0.964 |

[1]) mathematical model used in parenthesis [2]) number of contributing values [3]) standard error of the residuals
[4]) mean values with standard deviation in parenthesis [5]) t-value (paired t-test),
* significance of the bias $\bar{x} - \bar{y}$ ($p < 0.05$)

Tab. 4. Comparison of the results obtained by the EMIT digoxin assay mechanized with a ⟨Cobas⟩ Bio centrifugal analyzer and an EMIT/LAB system and by a radioimmunoassay in samples from patients. Various mathematical models were used for evaluation of the results obtained by the ⟨Cobas⟩ Bio.

| Methods (y vs x) | | n[2] | Standardized principal component Slope | Intercept | $s_{y.x}$[3] | $\bar{y}$ (s)[4] | $\bar{x}$ (s) | t[5] | Correlation coefficient |
|---|---|---|---|---|---|---|---|---|---|
| ⟨Cobas⟩ Bio (1)[1] | vs EMIT/LAB | 48 | 0.94 | − 0.05 | 0.33 | 1.84 (1.37) | 2.02 (1.46) | 2.47* | 0.939 |
| ⟨Cobas⟩ Bio (2) | vs EMIT/LAB | 48 | 0.91 | − 0.02 | 0.33 | 1.81 (1.33) | 2.02 (1.46) | 2.67* | 0.934 |
| ⟨Cobas⟩ Bio (3) | vs EMIT/LAB | 48 | 0.90 | 0.00 | 0.35 | 1.81 (1.31) | 2.02 (1.46) | 2.59* | 0.926 |
| ⟨Cobas⟩ Bio (4) | vs EMIT/LAB | 48 | 0.91 | − 0.02 | 0.32 | 1.81 (1.33) | 2.02 (1.46) | 2.73* | 0.937 |
| ⟨Cobas⟩ Bio (5) | vs EMIT/LAB | 48 | 0.89 | 0.00 | 0.33 | 1.79 (1.30) | 2.02 (1.46) | 2.89* | 0.932 |
| ⟨Cobas⟩ Bio (1) | vs Radioimmunoassay | 50 | 1.19 | − 0.08 | 0.22 | 1.78 (1.38) | 1.57 (1.16) | − 3.97* | 0.970 |
| ⟨Cobas⟩ Bio (2) | vs Radioimmunoassay | 50 | 1.15 | − 0.05 | 0.23 | 1.75 (1.34) | 1.57 (1.16) | − 3.52* | 0.965 |
| ⟨Cobas⟩ Bio (3) | vs Radioimmunoassay | 50 | 1.14 | − 0.02 | 0.24 | 1.75 (1.32) | 1.57 (1.16) | − 3.57* | 0.964 |
| ⟨Cobas⟩ Bio (4) | vs Radioimmunoassay | 50 | 1.16 | − 0.06 | 0.23 | 1.75 (1.34) | 1.57 (1.16) | − 3.55* | 0.966 |
| ⟨Cobas⟩ Bio (5) | vs Radioimmunoassay | 50 | 1.13 | − 0.04 | 0.24 | 1.73 (1.31) | 1.57 (1.16) | − 3.13* | 0.961 |
| EMIT/LAB | vs Radioimmunoassay | 48 | 1.26 | − 0.01 | 0.38 | 2.02 (1.46) | 1.61 (1.16) | − 4.84* | 0.926 |

[1]) mathematical model used in parenthesis [2]) number of contributing values [3]) standard error of the residuals
[4]) mean value with standard deviation in parenthesis [5]) t-value (paired t-test),
* significance of the bias $\bar{x} - \bar{y}$ ($p < 0.05$)

Tab. 5. Comparison of the results obtained by the EMIT thyroxine assay mechanized with a ⟨Cobas⟩ Bio centrifugal analyzer and an ABA-100 and by a radioimmunoassay in samples from patients. Various mathematical models were used for evaluation of the results obtained by the ⟨Cobas⟩ Bio.

| Methods (y vs x) | | n[2] | Standardized principal component Slope | Intercept | $s_{y.x}$[3] | $\bar{y}$ (s)[4] | $\bar{x}$ (s) | t[5] | Correlation coefficient |
|---|---|---|---|---|---|---|---|---|---|
| ⟨Cobas⟩ Bio (1)[1] | vs ABA-100 | 50 | 1.06 | − 0.56 | 0.96 | 9.42 (4.54) | 9.43 (4.28) | 0.05 | 0.954 |
| ⟨Cobas⟩ Bio (2) | vs ABA-100 | 50 | 1.06 | − 0.50 | 0.98 | 9.45 (4.52) | 9.43 (4.28) | − 0.14 | 0.952 |
| ⟨Cobas⟩ Bio (3) | vs ABA-100 | 50 | 1.09 | − 0.72 | 0.99 | 9.51 (4.65) | 9.43 (4.28) | − 0.41 | 0.954 |
| ⟨Cobas⟩ Bio (4) | vs ABA-100 | 50 | 1.01 | − 0.08 | 0.94 | 9.46 (4.34) | 9.43 (4.28) | − 0.18 | 0.951 |
| ⟨Cobas⟩ Bio (5) | vs ABA-100 | 50 | 1.09 | − 0.74 | 0.99 | 9.50 (4.65) | 9.43 (4.28) | − 0.39 | 0.954 |
| ⟨Cobas⟩ Bio (1) | vs Radioimmunoassay | 50 | 0.91 | − 0.46 | 0.43 | 9.42 (4.54) | 10.72 (4.92) | 11.60* | 0.989 |
| ⟨Cobas⟩ Bio (2) | vs Radioimmunoassay | 50 | 0.92 | − 0.40 | 0.47 | 9.45 (4.52) | 10.72 (4.92) | 10.62* | 0.988 |
| ⟨Cobas⟩ Bio (3) | vs Radioimmunoassay | 50 | 0.94 | − 0.62 | 0.48 | 9.51 (4.65) | 10.72 (4.92) | 10.71* | 0.988 |
| ⟨Cobas⟩ Bio (4) | vs Radioimmunoassay | 50 | 0.88 | 0.01 | 0.43 | 9.46 (4.34) | 10.72 (4.92) | 9.74* | 0.988 |
| ⟨Cobas⟩ Bio (5) | vs Radioimmunoassay | 50 | 0.95 | − 0.63 | 0.51 | 9.50 (4.65) | 10.72 (4.92) | 10.34* | 0.987 |
| ABA-100 | vs Radioimmunoassay | 50 | 0.87 | 0.09 | 0.85 | 9.44 (4.28) | 10.72 (4.92) | 6.23* | 0.959 |

[1]) mathematical model used in parenthesis [2]) number of contributing values [3]) standard error of the residuals
[4]) mean value with standard deviation in parenthesis [5]) t-value (paired t-test),
* significance of the bias $\bar{x} - \bar{y}$ ($p < 0.05$)

## Practicability and Costs

The ⟨Cobas⟩ Bio centrifugal analyzer appears to be very well suited for the EMIT. The system shows a high flexibility and allows rapid drug determinations. A single phenytoin determination for example takes about 5 minutes. Within one run about 10 patient samples can be analyzed in duplicate.

The costs for reagents and technician time for the ⟨Cobas⟩ Bio are significantly lower than for the original procedure (EMIT/LAB) or determinations with the Eppendorf analyzer 5010 (Tables 6, 7). Using the ⟨Cobas⟩ Bio the costs for a phenytoin determination, for example, can be reduced by about 60–70% and those for a digoxin determination even by 85% (tabs. 6, 7).

In a recent study (10) the reagent consumption was further minimized by use of the Cobas Bio, so that 600 EMIT theophylline assays per kit were possible while maintaining both acceptable precision and accuracy.

## Discussion

Compared with other analytical systems the ⟨Cobas⟩ Bio in particular shows a high flexibility which facilitates the adaptation of EMIT assays.

In most cases a very good between-days precision was observed with the EMIT phenytoin, methotrexate and disopyramide assays (figs. 1–3). However, the EMIT digoxin assay and, at low concentrations (3.1 µg/dl), the EMIT thyroxine assay (figs. 4–5), showed a some-

Tab. 6. Comparison of the direct costs (DM) of a quantitative phenytoin determination by EMIT using an EMIT/LAB system, an Eppendorf analyzer 5010 and a ⟨Cobas⟩ Bio centrifugal analyzer (November 1981).

| Costs | EMIT/LAB | | Eppendorf analyzer 5010 | | ⟨Cobas⟩ Bio | |
|---|---|---|---|---|---|---|
| | n[a]) = 1 | n = 10 | n = 1 | n = 10 | n = 1 | n = 10 |
| Technician time[b]) | 9.00 | 1.92 | 12.00 | 1.50 | 6.00 | 0.90 |
| Reagents, standards, control sera, and pertinent supplies | 74.00 | 19.33 | 111.49 | 20.94 | 30.79 | 7.45 |
| Total | 83.00 | 21.25 | 123.49 | 22.44 | 36.79 | 8.35 |

[a]) n = number of specimens per series

[b]) costs per minute technician time 0.60 DM (9)

Tab. 7. Comparison of the direct costs (DM) of a quantitative digoxin determination by EMIT using an EMIT/LAB system and a ⟨Cobas⟩ Bio centrifugal analyzer (November 1981).

| Costs | EMIT/LAB | | | ⟨Cobas⟩ Bio | | |
|---|---|---|---|---|---|---|
| | n[a]) = 1 | n = 10 | n = 20 | n = 1 | n = 10 | n = 20 |
| Technician time[b]) | 51.00 | 5.76 | 3.21 | 9.00 | 1.20 | 1.20 |
| Reagents, standards, control sera, and pertinent supplies | 35.92 | 12.39 | 9.95 | 8.82 | 1.57 | 1.48 |
| Total | 86.92 | 18.15 | 13.16 | 17.82 | 2.77 | 2.68 |

[a]) n = number of specimens per series

[b]) costs per minute technician time 0.60 DM (9).

what lower precision with the ⟨Cobas⟩ Bio than has been reported for other systems and possibly other reagent lots (1, 4, 7, 8). Calibration curves of the EMIT phenytoin and methotrexate assays were stable for a period of at least one hour.

The results of the EMIT digoxin assay determined by the ⟨Cobas⟩ Bio correlated better with those of a radioimmunoassay than the values obtained by EMIT with the EMIT/LAB system (tab. 4). Deviations of more than 30% between the results of EMIT and radioimmunoassay occurred by use of the EMIT/LAB system in 7 and with the ⟨Cobas⟩ Bio only in 3 out of the same 50 patient samples. In all of these discrepant cases the values measured by EMIT were higher than those obtained by radioimmunoassay. The lower incidence of possible interferences with the EMIT digoxin assay adapted to the ⟨Cobas⟩ Bio may be due to the higher dilution of the sample in the reaction medium. Furthermore the results of the EMIT thyroxine assay determined by the ⟨Cobas⟩ Bio and the original procedure with an ABA-100 were in good agreement (tab. 5). The thyroxine values obtained by EMIT were on average about 12% lower than those measured by radioimmunoassay. Comparing the EMIT thyroxine assay with radioimmunoassay (tab. 5), the standard error of the residuals was lower with the ⟨Cobas⟩ Bio than with the ABA-100.

In comparison with the original EMIT technique the best mathematical method for the evaluation of the results appeared to be model 1. In addition model 4 yielded very good results for thyroxine. Since most EMIT procedures apply a four or five parameter logit model it is not surprising that evaluation by model 1 provided a better correlation than model 5 (spline approximation). This conclusion is based on the reagent lots used during this study. In summary it is concluded that the ⟨Cobas⟩ Bio is very well suited for the mechanization of the EMIT, as it allows rapid, reliable determinations with this technique and a considerable reduction in direct costs.

## References


1. Oellerich, M. (1980) J. Clin. Chem. Clin. Biochem. *18*, 197–208.
2. Oellerich, M., Külpmann, W. R., Haeckel, R. & Heyer, R. (1977) J. Clin. Chem. Clin. Biochem. *15*, 353–358.
3. Oellerich, M., Engelhardt, P., Schaadt, M. & Diehl, V. (1980) J. Clin. Chem. Clin. Biochem. *18*, 169–174.
4. Oellerich, M., Haindl, H. & Haeckel, R. (1979) J. Clin. Chem. Clin. Biochem. *17*, 483–488.
5. Cook, R. & Wellington, D. (1980) Data handling for EMIT® assays, Syva, Palo Alto, U.S.A.
6. Reinsch, C. H. (1967) Numer. Math. *10*, 177–183.
7. Rosenthal, A. F., Vargas, M. G. & Klass, C. S. (1976) Clin. Chem. *22*, 1899–1902.
8. Sun, L. & Spiehler, V. (1976) Clin. Chem. *22*, 2029–2031.
9. Haeckel, R. (1979) Rationalisierung des medizinischen Laboratoriums, 2nd edition, GIT-Verlag Darmstadt.
10. Ou, C. N., Frawley, V. L. & Buffone, G. J. (1981) J. Anal. Toxicol. *5*, 249–252.



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